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Original article

SIMPLE, SENSITIVE AND RELIABLE LC-DAD METHOD OF GEMIFLOXACIN DETERMINATION IN PHARMACEUTICAL DOSAGE FORMS

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Abstract

A sensitive, accurate, rapid, fully validated, reliable and easy method was developed for the quantification of gemifloxacin mesylate (GEM) using liquid chromatography (LC) with DAD detection. In this method; a reversed-phase RP-18 (X-Select, 250 x 4.6 mm ID x 5μ) column with a mobile phase of methanol–water (50:50; v/v), containing 15 mM phosphoric acid (pH 2.50) at 1.0 ml/min flow rate was used to separate GEM and internal standard (IS) with a detection of 272 nm. Granisetron was chosen as IS. Using these conditions, the retention times were obtained as 3.20 min for IS, 4.25 min for GEM.

All necessary validation parameters and system suitability test results were obtained in detail. Linearity was obtained in the concentration range between 0.25 and 20 μg/mL. The LOD and LOQ values are 0.004, 0.013 μg/mL, respectively. Also the interday and intraday precision of the method was found as 0.069 % and 0.101 % RSD values, respectively. Also the validated method applied for pharmaceutical formulations and the accuracy were found 99.96 %. The above findings showed the proposed method is simple and has advantage of allowing for fast analysis.

Key words: *Gemifloxacin, LC, Pharmaceutical dosage form, Determination, Validation.*

Gemifloksasin'in Farmasötik Dozaj Şekillerinden Tayini için Basit, Duyarlı ve Güvenilir bir SK-FDD Yöntemi

Gemifloksasin mesilat'ın (GEM) miktar tayini için duyarlı, doğru, hızlı, tamamen valide edilmiş, güvenilir ve basit bir foto diyot dizi (FDD) dedektörlü sıvı kromatografi (SK) yöntemi geliştirilmiştir. Bu yöntemde GEM ve iç standartın (IS) 272 nm deki tayini için bir ters faz RP-18 (X-Select, 250 x 4.6 mm ID x 5μ) kolonla birlikte 1.0 ml/dak akış hızında 15 mM fosforik asit içeren (pH 2.50) metanol-su (50:50; h/h) karışımı hareketli faz olarak kullanılmıştır. İç standart (IS) olarak Granisetron seçilmiştir. Bu koşullar kullanıldığında alıkonma süreleri IS için 3.20 ve GEM için 4.25 dakika bulunmuştur.

Gerekli bütün validasyon parametreleri ve sistem uygunluk testi sonuçları detaylı olarak elde edilmiştir. Derişim aralığı 0.25-20 μg/mL arasında doğrusaldır. Gözlenebilme sınırı ve tayin alt sınırı değerleri sırasıyla 0.004 ve 0.013μg/mL olarak bulunmuştur. Yöntemin gün içi ve günler arası kesinlik çalışmaları sonucunda % Bağıl standart sapmaları (BSS) sırasıyla % 0.069 ve % 0.101'dir. Valide edilen yöntem farmasötik dozaj formlarına uygulanmış ve geri kazanım sonucu % 99.96 olarak bulunmuştur. Bu sonuçlar da göstermektedir ki önerilen yöntemin basit ve hızlı bir analize imkan verdiği için avantajlı olduğunu göstermektedir.

Anahtar kelimeler: *Gemifloksasin, SK, Farmasötik dozaj şekli, Miktar tayini, Validasyon.*

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INTRODUCTION

Gemifloxacin (GEM) 7-[(4Z)-3-(aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (Figure 1) is a new fluroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase IV. It uses similar to those of ciprofloxacin. It is used for the treatment of respiratory and urinary tract infection. It is given orally, as the mesylate, for the treatment of community acquired pneumonia and acute bacterial exacerbations of chronic bronchitis. The compound has broad spectrum of activity against gram-positive and gram-negative bacteria (1-3). Doses are expressed in terms of the base; 399 mg of gemifloxacin mesylate is equivalent to about 320 mg of gemifloxacin. The usual dose is 320 mg once daily for 5 days in patients with bronchitis or for 7 days in those with pneumonia. The length of time it needs to be taken and the dosage will depend on how the patient responds and what bacterial infection they have.

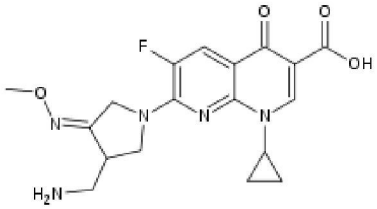

Figure 1. Chemical structure of GEM

In the open literature, few methods were reported for the quantitative determination of GEM in pharmaceutical dosage forms using voltammetric and spectrophotometric methods (4-6). A review of the literature revealed that limited high performance liquid chromatography (LC) methods have been reported for the determination of GEM in tablets (7-9) and stability indicating degradation analysis (10). The published LC assays have been described in the literature for the determination of GEM from pharmaceutical dosage form (7-9). However, the proposed procedure has lower determination limit and more precise when compared with the already published LC methods.

Because of their selectivity, sensitivity, and overall versatility, the development of reliable and validated HPLC methods has received considerable attention in the quality control of drugs and quantitative determination in pharmaceutical dosage forms. Owing to the widespread use of HPLC in routine analysis, it is important that good HPLC methods are developed and that these are thoroughly validated (11-15).

In the present study, simple, rapid, economical, sufficiently precise and accurate, reproducible, and fully validated RP-LC method with good detection ranges for estimation of GEM in pure form and in its solid dosage forms was developed. The proposed method was aimed at developing an easy and rapid assay method for GEM without any time-consuming sample preparation steps for routine analysis, to be adopted in quality control laboratories and, at the same time, ensure satisfactory recovery during drug estimation from pharmaceutical preparations. In the proposed RP-LC method, there is no need to extract GEM from the excipients matrix of pharmaceutical dosage forms, thereby decreasing the error in quantitation. The tablet dosage form samples can be directly used after dissolving, filtration, and then adequate dilution of supernatant liquid. The developed method was used to estimate the total drug content in commercially available tablet dosage forms of GEM. The results of the analysis were fully validated by statistics (16, 17) and recovery studies were realized.

EXPERIMENTAL

Equipment and chromatographic conditions

The Agilent Technologies HP 1100 series (Wilmington, DE) LC system was used for method development and validation studies, equipped with a G1379A degasser, G1311A quaternary pump, 61313A auto sampler, and G1315B diode array detector. The chromatograms were recorded and the peaks were quantified using an automatic integrator. The chromatograms were analyzed with Agilent Technologies HPLC 1100 software.

The separation was carried out at ambient temperature, on a reversed-phase Waters X-Select RP-18 column (250 x 4.6 mm ID x 5µ). The chromatographic separation was performed using an isocratic mode. The mobile phase consisted of a mixture of methanol–water (50:50; v/v), containing 15 mM phosphoric acid (pH 2.50). 1.0 mL/min flow rate was used for the separations with a detection of 272 nm. The injection volume was 10 µL. The chromatographic separation was performed at 25 ^{0}C. Granisetron was chosen as IS. The dead time (t_o) was measured by injecting urasil solution [0.01% (v/w), in water].

Chemicals and reagents

GEM reference substance and its tablet dosage form (Factive® Film tablet) were kindly supplied by Abdi Ibrahim Pharm. Ind. The reference substance of granisetron (internal standard) was kindly obtained from Mustafa Nevzat Pharm. Ind. Methanol was of HPLC grade, purchased from Merck (Darmstadt, Germany). All other chemicals were commercially analytical reagent grade. LC grade water was obtained following distillation in glass and passage through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all necessary solutions. Ortho-phosphoric acid (min. 85%) was from Riedel (Riedel-de Haen, Germany). Sodium hydroxide was purchased from Merck.

Working solutions were diluted with the mobile phase for preparing the necessary concentrations. All stock and working solutions were protected from light and stored in fridge at about 4 ^{0}C.

Standard solutions and calibration curves

The standard GEM and internal standard granisetron (IS) were prepared by dissolving 10 mg of compounds with 10 mL of methanol (MeOH) in a 10 mL volumetric flask. The concentration of GEM was varied in the range of 0.25 and 20 µg/mL and the concentration of IS was maintained at a constant level of 5.0 µg/mL. The appropriate dilutions were done using mobile phase. The calibration curve for HPLC analysis was constructed by plotting the ratio of the peak area of the drug to that of internal standard against the drug concentration. The linearity plots were constructed and the acceptable fit to the linear regression was demonstrated and reported by the necessary parameters.

All solutions were protected from light and were used within 24 h to avoid decomposition.

Analysis of tablets

Ten tablets labeled to contain 320.0 mg of GEM and excipients were weighed and finely powdered. An accurate weight of the powder equivalent to one tablet content was weighed, transferred into a 100 mL calibrated flask, diluted with methanol, stirred for about 10 min and then completed to volume with the same solution. This solution was filtered and the filtrate was collected in a clean flask. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate solution. After addition of the constant amount of IS (5.0 µg/mL), the solutions were diluted with mobile phase, in order to obtain a final solution. The content amount of GEM was calculated from its regression equation.

Ruggedness, precision, and accuracy

The ruggedness, intra-day and inter-day precision, and accuracy of the method were estimated by assaying five replicate samples at three different concentrations, on the same day and different days over a week period. The relative standard deviations (RSDs) were calculated to check the ruggedness and precision of the method (16, 17).

To determine intra-day precision (repeatability), six injections of the selected concentrations were given on the same day and the values of relative standard deviation were calculated. These studies were repeated with different concentrations on different days to determine inter-day precision (reproducibility).

To verify the accuracy of the method, recovery experiments were performed after addition a known amounts of pure drugs to pre-analyzed tablets. Known amounts of the pure drugs and a constant level of IS were added to the tablet formulation and the mixtures were analyzed. The percent recovery was calculated by comparing the concentration obtained from spiked samples with the actual added concentration. Thus, the effect of common excipients in tablet formulation on chromatograms (e.g., tailing, broadening) and the spectra was investigated. Recovery experiments from tablets also showed the reliability and suitability of the method. The accuracy was expressed as percentage bias (16, 17).

The resolution factor of the drug peak from the IS peak was also determined. Also all necessary system suitability test parameters were reported.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Precision and accuracy can often be enhanced by the use of an appropriate internal standard for an HPLC method, which also serves to correct for fluctuations in the detector response. One of the main reasons for using an internal standard is for samples requiring significant pretreatment or preparation. Often, a sample preparation step that includes reaction, filtration, precipitation, extraction, and so on, results in sample losses. When added prior to sample preparation, a properly chosen internal standard can be used to correct for these sample losses. The internal standard is a different compound from the analyte, but one that is well resolved in the separation. The chemical structure of granisetron is not similar to the GEM structure. However, it was chosen as the internal standard because it showed a shorter retention time with better peak shape and better resolution, compared with other potential internal standards.

To develop a rugged and suitable LC method for the quantitative determination of GEM, various mobile phase compositions and ratios were employed. Our preliminary trials using different compositions of mobile phases consisting of water, methanol, and acetonitrile, and also different ratios of these solutions, did not give good peak shape. After addition of 15 mM H_3PO_4 (pH 2.50) instead of acetonitrile improved the peak shape of GEM. Finally, by fixing the mobile phase composition consisting of a mixture of methanol:water (50:50 ;v/v), containing 15 mM phosphoric acid (pH 2.50), GEM and IS were resolved to the baseline and obtained the best peak shape. This mobile phase composition was found to be optimal for good peak shape as well as to achieve minimal background current. A reversed-phase Waters X-Select RP-18 (250 x 4.6 mm ID x 5µ particle size) was the most suitable column for LC analysis of GEM. Flow rate of 1.0 mL/min was selected for further studies after several preliminary investigatory chromatographic runs. Finally, using the conditions selected earlier, a satisfactory chromatographic peak resolution was obtained in a short analysis time, as can be seen in Figure 2. For GEM and IS, sharp and symmetrical single peaks were obtained with good resolution as well as to achieve minimal background current. The chromatographic separation was performed at 25 °C. Using the optimized operating conditions, the retention times were obtained as 3.20 min for IS, 4.25 min for GEM, being extremely stable among injections. Under the described experimental conditions, the GEM and IS peaks were well defined and free from tailing.

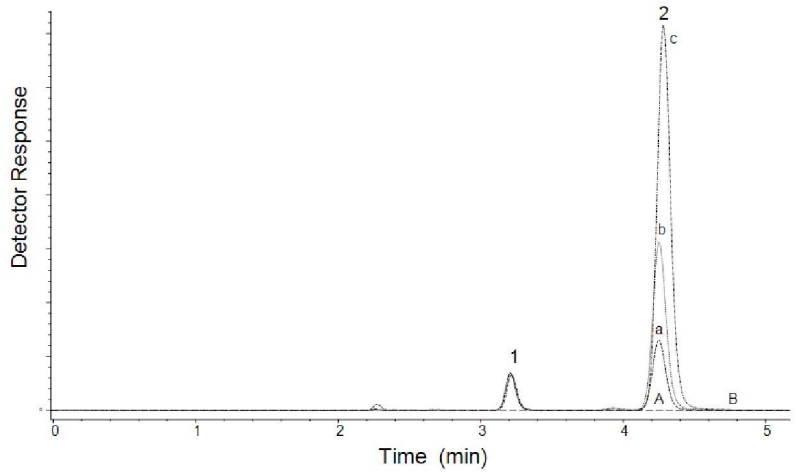

Figure 2. Chromatogram obtained from the mobile phase (A) and the standard solution (B) of (1) 5 µg/mL GRA (IS), (2) 2 µg/mL (a), 5 µg/mL (b), 12 µg/mL (c) GEM.

The proposed RP-LC method provided a simple procedure for the determination of the concentrations of GEM and IS in drug formulations by DAD detection at 272 nm. After determining the optimum conditions, a satisfactory resolution was obtained in a short analysis time (less than 5.0 min.).

Method validation

According to USP 24, method <621> (18) system suitability tests are an integral part of a liquid chromatographic method. A system suitability test can be defined as a test to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually designed after method development and validation have been completed. The parameters include tailing factor, retention factor, theoretical plate number, retention time, asymmetry factor, selectivity and RSD% of the peak height or area for repetitive injections. Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method. These tests were carried out on freshly prepared standard stock solutions of GEM. System suitability test results were reported in Table 1. According to Table 1, the results obtained from the system suitability tests satisfy the USP requirements.

Table 1. System suitability parameters of the proposed RP-LC method.

Parameters	IS	GEM	Recommended Value
Retention time (min)	3.200	4.250	
Resolution (R_s)	-	11.48	>2
Theoretical plates (N)	26102	27426	>2000
Selectivity factor (α)	-	2.72	>1
Tailing Factor	0.981	0.983	<2
RSD% (for retention time)	0.118	0.087	≤ 1

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve (16, 17, 19). The calibration curve was linear in the range of 0.25 and 20 µg/mL. The calibration curve equation is y=mx+n, where y represents the ratio of GEM peak area to IS peak area and x represents GEM concentration. Table 2 represents the calibration characteristics for the ratio of the peak areas of different amounts of GEM to a constant level of IS (5.0 µg/mL). The linearity of the calibration plots was confirmed by the high value of the correlation coefficient of GEM (Table 2). The injection volume was 10 µL.

The limit of detection (LOD) and quantitation (LOQ) of the procedure was also shown in Table 2. The low SE values of the slope and the intercept show the precision of the proposed method. The LOQ was determined as the lowest amount of analyte.

Table 2. Statistical evaluation of the calibration data of GEM by HPLC.

	GEM
Retention Time (min)	4.250
Linearity Range (µg/mL)	0.25-20
Slope	1.211
Intercept	0.017
Correlation Coefficient	0.999
SE of slope	$6.47x10^{-3}$
SE of intercept	$6.19x10^{-2}$
Limit of Detection (µg/mL)	0.004
Limit of Quantification (µg/mL)	0.013
Within-day Precision (RSD%)*	0.069
Between-day Precision (RSD%)*	0.101

* Each value is the mean of five experiments.

The stability of the reference substance and sample solutions were checked by analyzing a prepared standard solution of GEM in mobile phase aged at $+4^0$C, in the dark against a sample freshly prepared. The results demonstrated that the working reference solutions were stable for up to 2 weeks. The GEM area ratio to IS for the assay reference solutions over a week period did not change considerably.

Precision

The intra- and inter-day variability or precision data are summarized in Table 2. They were assessed by using standard solutions prepared to produce solutions of different concentrations of each drug in the mobile phase. Repeatability and reproducibility were characterized for different concentrations and given by mean recovery and RSD% (Table 2). Based on these results, there was no significant difference for the assay, as tested by within-day (repeatability) and between days (reproducibility). Repeatability or intra-day precision was investigated by injecting five

replicate assays of the samples. Inter-day precision were assessed by injecting the samples over three consecutive days.

Assay

When working on raw material, results encourage the use of the proposed method described for GEM in commercial tablet dosage forms. The results, corresponding to the tablet dosage form of GEM, are shown in Table 3. Proposed RP-LC method can be used for the determination of GEM without prior separation of the excipients. Each tablet contains the active ingredients which is 320.0 mg of GEM and the inactive ingredients. Removal of the excipients before analysis was found to be unnecessary. Fig. 3 shows a typical chromatogram obtained follow by the analysis of GEM in tablets with IS. As shown in Fig. 3, the substances were eluted, forming well shaped, symmetrical single peaks, well separated from the solvent front. No interfering peaks were obtained in the chromatogram from the tablet excipients. The labeled amount of drugs indicates that the active ingredients in samples were present at a level included within the requirements with respect to the label claimed by the manufacturer. The utility of all of the proposed method was verified by means of replicate estimations of pharmaceutical preparations and results obtained were evaluated statistically (Table 3).

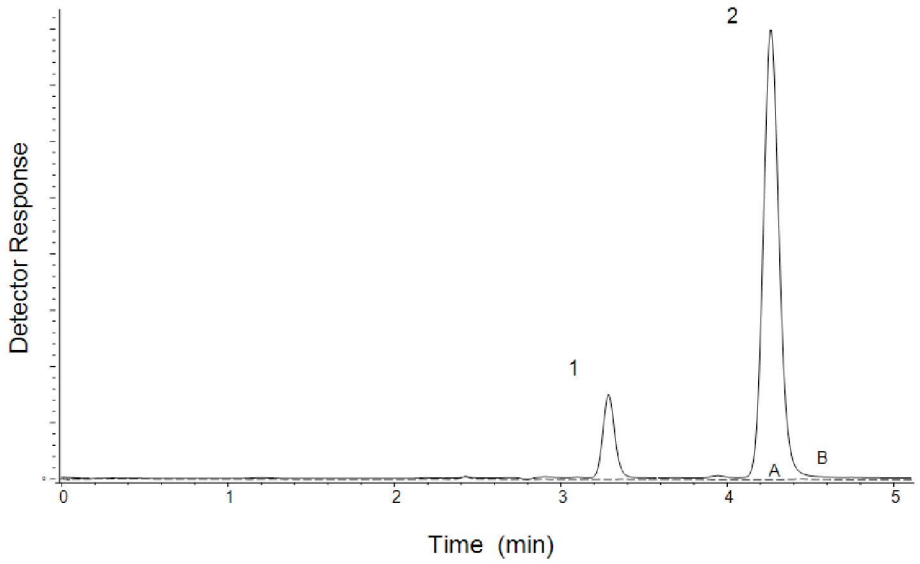

Figure 3. Chromatogram obtained from the mobile phase (A) and pharmaceutical dosage form (B); containing: (1) 5 µg/mL GRA (IS), (2) 6 µg/mL GEM.

Results obtained from proposed method of the analysis of GEM in tablets indicate that the proposed technique can be used for quantitation and routine quality control analysis of GEM in pharmaceutical dosage forms. A comparison with an official reference determination method has not yet been possible neither in any pharmacopoeia nor the literature, since no other procedure for the determination of GEM from pharmaceutical formulations has been reported so far.

Table 3. Results of the assay and the recovery analysis of GEM in pharmaceutical dosage form.

	GEM
Labeled claim (mg)	320.00
Amount found (mg)*	319.71
RSD (%)	0.159
Bias (%)	0.089
Added (mg)	9.000
Found (mg)*	8.997
Recovery (%)	99.96
RSD % of recovery	0.149
Bias (%)	0.036

* Each value is the mean of three experiments.

Accuracy

In order to demonstrate the accuracy, validity, and applicability of the proposed LC method, recovery tests were carried out. The recovery of the procedure was carried out by spiking already analyzed samples of tablets with the known concentrations of standard solutions of GEM. The recovery results for all techniques are shown in Table 3. In each case, the relative error (Bias %), RSD %, and accuracy was calculated for each of the compounds.

It is concluded that the proposed methods are sufficiently accurate and precise in order to be applied to pharmaceutical dosage forms. Using this proposed method, high percentage recovery data was obtained. The results of the recovery assay indicate that the method is selective, accurate and precise for the analysis of GEM without interference from the excipients used to formulation and produce the tablet dosage form. High percentage of recovery shows that the method is free from the interferences of the excipients used in the formulations.

CONCLUSIONS

An analytical RP-LC method developed and validated for the quantitative determination of GEM in bulk and as well as in pharmaceutical dosage forms has been described. The proposed method gives good resolution between GEM and IS within a short analysis time. The proposed method was faster, more precise and accurate when compared with the already published methods (7-9), hence it is more suitable for the routine analysis. The RP-LC method developed in this study has the advantage of simplicity, precision, and reliability. It allows for the direct determination of GEM, by passing several tedious steps. There was no significant difference for the assay tested within day and between days. The proposed method was applied to the direct determination of GEM in tablet formulation. The method developed would serve as a versatile analytical tool suitable for the analysis of GEM and would be of interest for quality control and clinical monitoring laboratories. High percentage of recovery results shows that the proposed

method is free from the interferences of the commonly used excipients and additives in the formulations of the drug.

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